

# 苹果与胶孢炭疽菌互作研究进展<sup>1</sup>

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**摘要:** 胶孢炭疽菌 (*Colletotrichum gloeosporioides*) 能够引发苹果苦腐病、苹果果实炭疽病及炭疽叶枯病, 危害叶片和果实, 影响果品产量和品质, 给苹果产业造成严重的经济损失。本文对苹果与病原物互作分子机制最新研究进展进行综述, 包括苹果上炭疽病的病原菌组成、分类、侵染循环及其引发的果树病害种类; 病原菌的致病结构和降解酶类、致病相关基因的挖掘与分析、效应蛋白的筛选与功能分析等致病相关分子机理; 苹果被侵染后生理生化变化、激素信号、抗病基因挖掘、MiRNA 参与的免疫调控机制等抗病机制研究进展。为解析病原菌致病机制及与寄主互作机制, 进而为挖掘潜力候选基因, 为病害综合防控和抗病分子育种奠定理论基础。

**关键词:** 苹果; 胶孢炭疽菌; 侵染机制; 互作机制

中图分类号: S661.1 文献标志码: A 文章编号: 1009-9980(2024)06-0001-08

## Advances in studying the relationship between Apple and

### *Colletotrichum gloeosporioides*

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**Abstract:** *Colletotrichum gloeosporioides* can cause apple bitter rot, apple fruit anthracnose, and anthracnose leaf blight, endangering leaves and fruits, affecting fruit yield and quality, and causing serious economic losses to the apple industry. According to the latest fungal classification system, the *Colletotrichum gloeosporioides* species complex consists of 13 different species, including *C. gloeosporioides*, *C. aenigma* and *C. fructicola* et al. Among them, *C. fructicola* and *C. gloeosporioides* are important pathogenic fungi on various fruit trees. Meanwhile, *C. gloeosporioides* can also cause fruit tree diseases such as cherry, passion fruit, and kiwifruit. In order to better prevent and control diseases, we need to have a comprehensive understanding of the classification, pathogenic mechanisms, and host interaction mechanisms of pathogens on apples. In the process of colonizing host tissue, a number of *C. gloeosporioides* genes participate

收稿日期: 2024-03-13 接受日期: 2024-04-07

基金项目: 中央级公益性科研院所基本科研业务费专项 (1610182023012); 国家现代农业产业技术体系资助 (CARS-27); 中国农业科学院科技创新工程专项经费项目 (CAAS-ASTIP-2021-RIP-05)。

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in different phases of infection procedures, which include conidiation, appressorium morphogenesis, melanization and penetration, biotrophy, necrotrophy, and various transport activities. In recent years, research on the pathogenic molecular mechanism of *C. gloeosporioides* on apples has mainly focused on the cloning and analysis of pathogenic related genes, screening and identification of effector proteins, pathogenic enzymes, and collettotoxins of *C. gloeosporioides*. Fungi secrete enzymes such as pectin, keratin and cellulase to help them successfully infect their hosts. New studies has shown that the adapter protein gene *GcAPI* can regulate the expression of endopolygalacturonase genes (*CgPG1* and *CgPG2*), pectin lyase genes (*pnl-1*, *pnl-2*), and pectate lyase genes (*pelA*, *pelB*), *GcAPI* is an important virulence factor of *C. gloeosporioides*. Currently, the successful application of PEG mediated genetic transformation and *Agrobacterium mediated transformation* in the study of *C. gloeosporioides* provides a theoretical basis for the development of pathogenic molecular mechanisms. It has been confirmed that genes with different functions such as *CgABCF2*, *CgCMK1*, *CgSET5*, *CgOpt1*, *CgNVF1*, *CgABCF2*, *CgChip6* are present in *C. gloeosporioides*, playing an important role in infecting apples. In addition, C2H2 transcription factors, cation stress response transcription factors *CgSltA*, *CgCrzA*, and *CsHtf1* also play important roles in pathogen pathogenesis. During the infection process, *C. gloeosporioides* can also secrete a series of effectors to inhibit the host immune response, thereby promoting pathogen infection and colonization. Currently, scientists have analyzed the roles of effectors such as *CfE12*, *CfEC92*, and *Sntf2* in *C. gloeosporioides*, laying the foundation for subsequent research on pathogen host interactions. In addition, *C. gloeosporioides* secrete toxins during the necrotrophic stage, causing necrosis of the host tissue. The research on apple disease resistance started relatively late, mainly focusing on germplasm resource identification, physiological and biochemical testing, disease resistance gene mining, plant hormone mediated disease resistance response, disease related transcription factors, and other mechanisms of action. Research has shown that after inoculation with anthrax fungus, the activities of superoxide dismutase (SOD), polyphenol oxidase (PPO), peroxidase (POD), catalase (CAT), and serotonin N-acetyltransferase (SNAT) in apple leaves increased, indicating that these enzymes are involved in the infection process of *C. gloeosporioides*. Plant hormones play an important role in plant defense and growth and development, and hormones related to plant immune responses include salicylic acid (SA), jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), and so on. Research has shown that there are significant differences in the expression levels of SA synthesis related genes *MdEDS1*, *MdPAD4*, *MdPAL* and SA signal transduction related genes *MdNPR1*, *MdPRI* and *MdPR5* between resistant and susceptible varieties. There are differences in the resistance and susceptibility of different apple varieties to *C. gloeosporioides*. The 'Hanfu' variety has been used to screen for resistance genes due to its high resistance to *C. gloeosporioides*. WRKY and NAC transcription factors play a crucial role in plant resistance to pathogen infection. In apples, transcription factors *MdWRKY15*, *MdWRKY17*, and *MdWRKY100* enhance apple resistance to anthracnose by regulating SA accumulation. Here, we plotted the downstream regulatory patterns

of AtwvKY33 and MdWRKYs involved in the MAPK cascade reaction, and presented some research results on MdWRKYs. At the end of the article, we summarized the research results on the regulatory mechanism of miRNA involvement in plant immunity. Clarifying the pathogenic process and molecular mechanism of the pathogen is of great significance for the comprehensive prevention and control of *C. gloeosporioides*. With the deepening of various studies, researchers will inevitably change their thinking on the prevention and control of *C. gloeosporioides* poses a huge threat to the apple industry. Traditional chemical prevention and control methods, such as the extensive use of fungicides and insecticides, can achieve the effect of combating pathogens, but they also cause serious harm to the environment and people. Carrying out the breeding of resistant varieties is a fundamental means to solve the difficulties in preventing and controlling *C. gloeosporioides*. This article aims to analyze the pathogenic mechanism of pathogens and their interaction with hosts, laying a theoretical foundation for screening potential candidate genes and disease resistant molecular breeding.

**Key words:** Apples; *C. Gloeosporioides*; Infection mechanism; Interaction mechanism

炭疽菌 (*Colletotrichum*) 属小丛壳科刺盘孢属真菌, 有性型为子囊菌门盘菌亚门小丛壳属, 在温暖和潮湿的条件下易爆发流行, 是世界上重要的植物病原菌之一<sup>[1]</sup>。炭疽菌可分为14个复合种和部分种, 胶胞炭疽菌 (*C. gloeosporioides*) 是重要的一种, 能侵染1000余种作物, 危害枝干、叶部、果实等部位, 造成果实腐烂、植株枯萎甚至死亡。

*C. gloeosporioides*通过“半活体营养”寄生并侵染寄主植物<sup>[2]</sup>, 在整个侵染周期主要有活体营养型 (biotrophic) 和死体营养型 (necrotrophic) 两种营养模式。在侵染初期活体营养阶段菌体不会立即杀死周边寄主细胞, 而是感应寄主表面的物理和化学信号 (植物表面硬度、疏水性、叶片纹理、植物激素等), 产生初侵染菌丝摄取寄主体内营养和能源。后期, 分化出初生菌丝和次生菌丝并迅速扩展, 分泌细胞壁降解酶导致植物组织形成坏死斑, 后转换为死体营养<sup>[3-5]</sup>, 其生活史和侵染过程如 (图1) 所示。

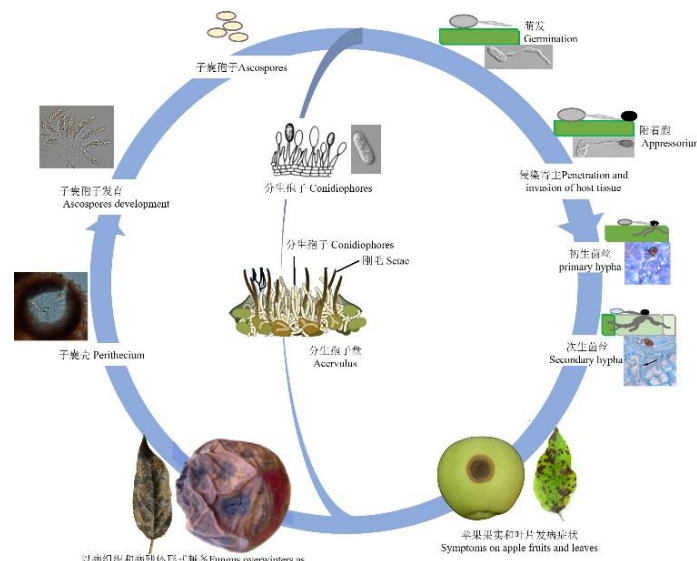


图1 胶孢炭疽菌生活史及其侵染过程示意图

Fig.1 Schematic diagram of the disease cycle and infection process of *Colletotrichum gloeosporioides*

目前，生产上对炭疽菌的防控以化学农药为主，随着人们对果品安全的逐渐重视，科研者开展了药剂筛选和复配<sup>[6]</sup>、农药助剂应用<sup>[7]</sup>及植物免疫诱抗剂使用等<sup>[8]</sup>药剂减量增效研究。对苹果炭疽病菌的侵染和致病研究取得一定进展，但因其种群多样、侵染过程复杂，对其侵染致病机制和果树抗性机制的研究仍有待深入。本文将围绕苹果胶孢炭疽菌病原学、病原菌致病机制及果树抗性机制开展论述。

### 1. 侵染苹果的胶孢炭疽菌复合群概述

胶孢炭疽菌 (*Colletotrichum gloeosporioides*) 能够引发苹果果实炭疽病 (Apple bitter rot) 和苹果炭疽叶枯病 (Glomerella leaf spot of Apple, GLSA)，也能引发果实采后炭疽病。胶孢炭疽菌复合种 (*C. gloeosporioides* complex) 是苹果上主要的病原菌，包含果生刺盘孢 (*C. fructicola*)、隐秘刺盘孢 (*C. aenigma*)、胶孢刺盘孢 (*C. gloeosporioides*) 等13种不同炭疽菌，其中*C. fructicola*和*C. gloeosporioides*是多种果树的重要致病菌，可以在无伤条件下成功侵染寄主<sup>[9,10]</sup>。除苹果外，*C. gloeosporioides*还可引发樱桃、百香果、猕猴桃等果树病害<sup>[10-15]</sup>，见表1。

表 1 近 5 年来有关胶孢炭疽菌侵染果树的报道

Table1 Report on the infection of fruit trees by *C. gloeosporioides* in the past 5 years

病原菌 Name of Fungi	树种 Host	参考文献 References
果生刺盘孢 <i>C. fructicola</i>	蛋黄果、樱桃、软枣猕猴桃、石榴、百香果 <i>Pouteria campechiana (Kunth) Baehni</i> 、 <i>Prunus avium</i> 、 <i>Actinidia arguta</i> 、 <i>unica granatum L.</i> 、 <i>Passiflora edulis</i>	[16-19]
隐秘刺盘孢 <i>C. aenigma</i>	苹果 <i>Malus domestica</i>	[20]
暹罗刺盘孢 <i>C. siamense</i>	木瓜、苹果 <i>Carica papaya L.</i> 、 <i>Malus domestica</i> ,	[21]
胶孢刺盘孢 <i>C. gloeosporioides</i>	葡萄 <i>Vitis vinifera L</i>	[22]
异国刺盘孢 <i>C. alienum</i>	芒果 <i>Mangifera indica L.</i>	[23]
亚洲刺盘孢 <i>C. asianum</i>	苹果 <i>Malus domestica</i>	[24]

### 2. 胶孢炭疽菌致病分子机理

随着生物信息学和分子生物学的发展，胶孢刺盘孢 (*C. gloeosporioides*)、希金斯刺盘孢 (*C. higginsianum*)、禾生刺盘孢 (*C. graminicola*)、东方刺盘孢 (*C. orbiculare*) 等全基因组测序组装完成并公布。在此基础上，PEG 介导的遗传转化<sup>[25]</sup>和农杆菌介导的转化<sup>[26]</sup>在苹果炭疽菌研究中成功应用，为病原菌致病分子机制的开展提供了理论依据<sup>[22,23]</sup>。近年来苹果上胶孢炭疽菌致病分子机理的研究主要集中在致病结构和降解酶测定、致病基因的克隆与分析、效应蛋白筛选与功能研究及炭疽菌毒素等方面<sup>[27-29]</sup>。

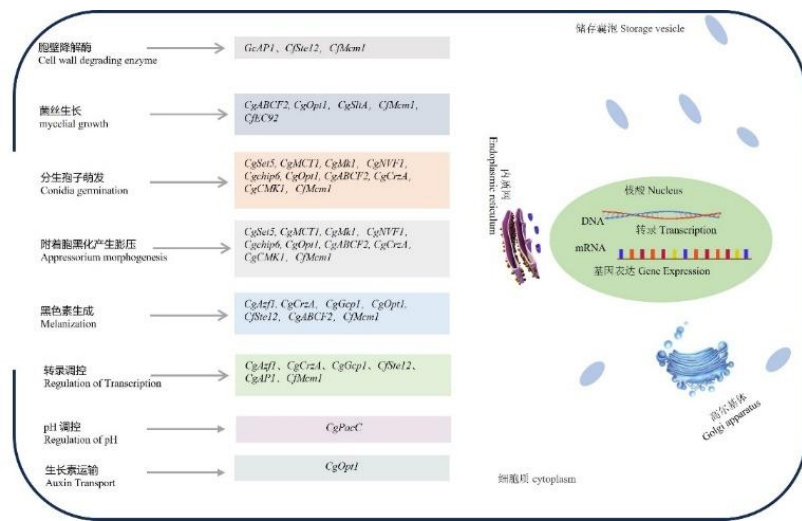


图2 苹果中已报导的胶孢炭疽菌致病相关基因

Fig.2 Pathogenic genes related to *C. gloeosporioides* in apple trees

### 2.1 胶孢炭疽菌致病结构和降解酶

炭疽菌要穿透寄主的表皮组织，需要对寄主组织施加机械压力，即孢子萌发时形成的附着胞及其胞内组合液产生膨压，压力施加至附着胞下部的侵染钉，当压力到达一定程度时直接穿透植物表皮而侵入，构成侵染和定殖<sup>[30]</sup>。黑化附着胞的形成可能涉及一系列复杂的生物学过程，包括分泌特定的分子、改变细胞壁结构以提供附着支持等<sup>[31]</sup>。

植物的细胞壁是抵御病原菌侵入的天然屏障，病原菌通过分泌产生果胶酶、角质酶、纤维素酶、蛋白酶等降解酶类物质破坏寄主细胞壁辅助其侵染和定殖。薛莲对苹果采后炭疽病菌细胞壁降解酶活性分析，明确聚甲基半乳糖醛酸酶（PMG）和羧甲基纤维素酶（Cx）在病原菌侵染中发挥重要作用<sup>[32]</sup>。研究表明衔接蛋白GcAP1复合体分布于细胞质中，GcAP1基因能够调控多聚半乳糖醛酸内切酶基因（CgPG1、CgPG2）果胶裂解酶基因（*pnl-1*、*pnl-2*）果胶酸酯裂解酶基因（*pelA*、*pelB*）的表达，从而影响炭疽菌的生长发育和毒力<sup>[33]</sup>。研究表明，胶孢炭疽菌pH依赖性转录因子CgPacC能够调节细胞壁降解酶、转运蛋白和抗氧化剂的表达，在病原菌定殖中发挥着重要作用<sup>[34]</sup>。

### 2.2 胶孢炭疽菌侵染阶段相关致病基因挖掘与分析

*C. gloeosporioides*成功侵染定殖包括分生孢子萌发、附着胞形成、黑色素生成、侵染钉穿透寄主组织等不同阶段，涉及的基因及其调控机制较为复杂，目前的研究以单一基因为主。

Zhao等证实苹果炭疽叶枯病菌染色质调节剂CgSET5在菌丝生长、分生孢子形成、附着胞形成、细胞壁完整性、致病性中发挥着重要作用，并同时参与过氧化物酶体的生物反应，是*C. gloeosporioides*的核心致病调节因子<sup>[35]</sup>。在后续研究中，也证实单羧酸转运蛋白

CgMCT1参与了*C. gloeosporioides*营养生长、黑色素形成、分生孢子形成，且参与寄主体内ROS降解<sup>[27]</sup>。Zhou等发现当炭疽菌CgABC2基因缺失后，菌丝生长速率和附着胞数量显著下降，导致致病性丧失<sup>[28]</sup>。张俊祥等研究证实CgCMK1、CgNVF1在炭疽菌分生孢子和附着胞中的表达，对分生孢子产量、附着胞形成、氧化胁迫应答反应及致病性等方面均有影响<sup>[36, 37]</sup>。徐杰证实苹果炭疽叶枯病菌基因GTPBP1在调控附着胞的形成中发挥着作用<sup>[38]</sup>。谭清群研究证实氨甲酰磷酸合成酶（Carbamyl phosphate synthase, CPS）小亚基基因Cpa1通过调控精氨酸的合成从而影响病原菌致病力<sup>[39]</sup>。研究表明寡肽转运蛋白基因CgOpt1在菌丝中表达，参与了真菌对IAA反应的调节，通过影响产孢和色素沉积来降低病菌的致病性<sup>[40]</sup>。甾醇糖基转移酶编码基因CgChip6参与分生孢子萌发和附着胞的形成，该基因缺失后病原菌毒力的显著下降<sup>[41]</sup>。Liang等对果生炭疽菌（*C. fructicola*）1104-7基因组进行了测序和组装，获得了高质量参考基因组，为*C. fructicola*致病相关基因的研究提供了重要的理论和数据支撑<sup>[42]</sup>。

### 2.3 转录因子调控胶孢炭疽菌分子机制

转录因子（transcription factor, TF）能够与基因自动自区域的顺式作用元件特异性互作，从而调控目的基因的表达强度，可分为4类，即锌指蛋白（包括3类：C2H2、C4和C6）、碱性亮氨酸拉链、碱性螺旋环螺旋和同源异形盒类转录因子<sup>[43]</sup>。已有研究表明，胶孢炭疽菌的转录因子基因在表达调控中能起到协调作用，能够促进附着胞黑化和定殖。C<sub>2</sub>H<sub>2</sub>锌指蛋白型转录因子CgAzf1、CgCrzA及CgGcp1能够调控黑色素生物合成途径相关基因的表达，参与分生孢子的萌发和侵染过程<sup>[31, 44, 45]</sup>。CfSte12能够调控与附着胞功能相关的四次穿膜蛋白PLS1（tetraspanin PLS1）、Gas1样蛋白（Gas1-like proteins）、角质酶和黑色素合成的基因表达<sup>[46]</sup>。阳离子胁迫反应转录因子CgSltA、CgCrzA及CsHtf1在炭疽菌营养生长、分生孢子产生、附着胞形成和致病性等方面均发挥重要作用<sup>[45, 47]</sup>。碱性亮氨酸拉链（Basic leucine zipper, bZIP）转录因子CgAP1在*C. gloeosporioides*中起氧化还原传感器的作用<sup>[48, 49]</sup>。转录因子CfMcm1是*C. fructicola*的关键调节因子，在病原菌无性繁殖、黑色素形成、致病性、果胶酶降解等过程中发挥功能<sup>[50]</sup>。

### 2.4 效应蛋白筛选及功能研究

在侵染过程中，炭疽菌通过分泌一系列效应蛋白抑制寄主免疫反应，从而促进病原菌的侵染和定殖<sup>[51, 52]</sup>，不同侵染阶段所分泌的效应因子功能不同<sup>[3, 53]</sup>。随着基因组测序的应用，炭疽菌中多个候选的效应因子被成功筛选鉴定<sup>[54, 55]</sup>。真菌胞外膜蛋白CFEM（common in several fungal extracellular membrane）是真菌所独有的蛋白结构域，与病原菌致病性密切相关。Shang等研究证实，刺盘孢属真菌CFEM型效应因子CfEC12能够与苹果中MdNIMIN2互作，与竞争水杨酸受体NPR1竞争MdNIMIN2蛋白的结合位点，从而抑制苹果抗性基因的表达和免疫反应<sup>[56]</sup>。LysM型效应蛋白可以保护真菌细胞壁免受植物几丁质酶的作用或隔离释放的壳寡糖，从而避免被植物的防御系统识别。其具有几丁质结合活性，可以结合几丁

质从而抑制植物的PTI (Pattern-triggered immunity), 促进病原菌的侵染<sup>[57]</sup>。Shang等研究证实*C.fructicola*中效应蛋白CfEC92在早期附着胞生成和附着胞介导的渗透阶段上调表达可抑制苹果的PTI和相关防御基因表达, 促进病原菌侵染<sup>[58]</sup>。Wang等开展效应蛋白Sntf2功能研究, 证实其能够与叶绿体组装因子*Mdyef39*互作干扰叶绿体功能, 从而抑制寄主植物的免疫反应, 促进了苹果炭疽叶枯病菌的侵染和定殖<sup>[59]</sup>。

## 2.5 胶孢炭疽菌毒素

炭疽菌在死体营养阶段通过分泌毒素造成寄主组织坏死。目前, 对于炭疽菌毒素的研究多集中在毒素生物测定、成分鉴定纯化阶段。*C. gloeosporioides*产生的毒素为非寄主专化性毒素, 能够侵染多种寄主。Khodadadi等分离鉴定了苹果苦腐病病原, 明确其毒素能够对3种不同树种造成危害<sup>[60]</sup>, 关于炭疽菌的毒素和作用机理仍然有待进一步深入研究。

## 3 苹果抗胶孢炭疽菌侵染的分子机理

果树在自然环境中会受到各类病原物的侵染, 为了抵御病原菌的侵染, 植物进化出识别和抵御病原菌的PTI和ETI两层免疫系统<sup>[61]</sup>。第一层免疫系统是由植物细胞质膜上的模式识别受体感知微生物相关分子模式 (microbe-associated molecular pattern, MAMPs) 或损伤相关分子模式 (damage-associated molecular pattern, DAMPs) 而触发一系列的免疫反应, 称为“模式触发免疫”, PTI, 该免疫反应包括活性氧 (Reactive oxygen species, ROS) 的激活及抗病基因表达量上调等<sup>[62, 63]</sup>。病原菌为了应对植物的PTI免疫反应进化出毒力蛋白 (效应因子), 抑制植物PTI反应, 从而成功侵入, 这一中间过程被称为“效应因子触发的易感性”, 即EST (Effector-triggered susceptibility)。最后, 植物进化出识别和抵御这些效应因子的胞内NLR来诱导更为强大的抗性反应, 即第二层免疫“效应因子触发的免疫”, ETI (Effector-triggered immunity) <sup>[64, 65]</sup>。ETI的免疫反应主要包括程序性细胞死亡的过敏性反应 (Hypersensitive responses, HR)、Ca<sup>2+</sup>内流、胼胝质的沉积等。植物在PTI和ETI期间, 产生的免疫反应幅度和时间有所不同, 但所触发的免疫信号网络和下游反应有所重叠<sup>[66-68]</sup>。

苹果抗炭疽病的研究起步相对较晚, 主要开展了种质资源鉴定<sup>[69]</sup>、生理生化检测、抗病基因挖掘、植物激素介导的抗病反应及抗病相关转录因子<sup>[70]</sup>作用机制等的研究。

### 3.1 生理生化变化

研究表明接种炭疽菌后, ‘嘎拉’和‘富士’叶片内超氧化物歧化酶 (superoxide dismutase, SOD)、多酚氧化酶 (polyphenoloxidase, PPO)、过氧化物酶 (peroxidase, POD)、过氧化氢酶 (catalase, CAT)、5-羟色胺-N-乙酰基转移酶 (SNAT) 的活性增强, 其相关基因表达量呈先升后降趋势, 表明以上酶类参与了炭疽叶枯病菌的侵染过程<sup>[71, 72]</sup>。苹果不同组织被炭疽菌侵染后, 多酚氧化酶 (PPO)、过氧化物酶 (POD)、苯丙氨酸解氨酶 (PAL) 等7种酶活性均有不同程度地提高<sup>[73-75]</sup>。通过分析不同抗感品种感染炭疽菌后细胞壁降解酶活性的变化, 证实甲基半乳糖醛酸酶 (PMG) 和羧甲基纤维素酶 (Cx) 在病

菌侵染过程中发挥着作用，且抗病品种中细胞壁降解酶活性高峰的出现早于感病品种<sup>[76]</sup>。白静科等比较了*C. fructicola*侵染后抗感品种中过氧化氢（H<sub>2</sub>O<sub>2</sub>）和乳突产生的差异，发现炭疽菌的侵染诱导了苹果细胞中H<sub>2</sub>O<sub>2</sub>的积累和乳突的产生，并随着侵染时间不断积累<sup>[30]</sup>。此外，生防菌也可以通过提高感病品种‘嘎拉’叶片中POD、CAT、SOD等防御酶地活性，减少活性氧的积累，从而诱导苹果对炭疽菌的抗性<sup>[77]</sup>。

### 3.2 植物激素

植物激素在植物防御和生长发育中发挥着重要作用，与植物免疫反应相关的激素包括水杨酸（SA）、茉莉酸（JA）、乙烯（ET）、脱落酸（ABA）等。SA和JA-ET激素作为重要的调控因子在苹果生物和非生物胁迫反应中发挥着重要作用<sup>[78, 79]</sup>。SA是通过异分支酸合成酶（ICS）和苯丙氨酸解氨酶（PAL）途径合成。在应激条件下，超过90%的受刺激SA通过ICS合成的<sup>[80]</sup>。当没有遇到病原体或逆境时，植物细胞积累了相对较低浓度的SA，外源喷施SA可增强抗病相关酶的活性，诱导高感苹果品种对*C. gloeosporioides*产生抗性<sup>[81, 82]</sup>。苹果中，‘藤牧1号’、‘40-9’及‘16-16’等抗性品种（系）中SA合成相关基因*MdEDS1*、*MdPAD4*和*MdPAL*被*C. gloeosporioides*诱导表达，SA信号转导相关基因*MdNPR1*、*MdPR1*、*MdPR5*的表达显著高于‘嘎拉’等感病品种（系）<sup>[83]</sup>。水杨酸合成途径中的关键酶MdICS1可以被*G. cingulata*诱导上调表达，而JA、ABA和ETH三种外源信号可抑制其表达。

### 3.3 苹果抗病基因挖掘

不同苹果品种对炭疽菌抗感表现存在差异，在苹果炭疽叶枯病上田间表现尤为突出<sup>[84]</sup>。马玉鑫研究表明‘寒富’品种CDPK基因家族成员*MdCDPK24*基因在炭疽菌侵染后显著上调表达<sup>[85]</sup>。对‘寒富’苹果同源四倍体进行转录组测序，发现*MdCaMBP6*、*MdIPT8*在苹果炭疽叶枯病菌侵染后显著上调表达，能够提高品种抗性<sup>[86, 87]</sup>。GUO等报道湖北苹果*Malus hupehensis*YT521-B同源结构域包含蛋白2（MhYTP2），其与*MdRGA2L* mRNA结合并降低其稳定性，在调节对炭疽叶枯病的抗性中发挥着重要作用，可用于开发具有GLS抗性的苹果品种<sup>[88]</sup>。刘源霞等采用分离群体分组分析（BSA）方法筛选获得了一个与抗病性状相关的分子标记S0506206-24<sup>[89]</sup>，在此基础上，采用全基因组重测序和BSA相结合的方法在该杂交群体中定位了1个苹果抗炭疽叶枯病基因位点*Rgls*，并将其精细定位于标记InDel14199和SNP4299之间<sup>[90]</sup>，室内接种验证与*Rgls*位点紧密连锁的4个分子标记S0405127（SSR）、S0304673（SSR）、SNP4236和InDel4254，准确率均高于90%<sup>[91]</sup>。

### 3.4 抗病相关转录因子参与的防御反应

植物被病原物感染后，当病原体相关的分子模式（PAMP）或效应器被植物识别时，细胞内的信号可以被激活，导致活性氧簇（ROS）的产生、丝裂原激活的蛋白激酶（MAPK）激活和防御基因的表达<sup>[92]</sup>。MAPKs能够靶向并磷酸化调节下游基因转录的转录因子，最终响应病原菌的侵入。已报道的与炭疽菌侵染响应相关的转录因子有AP2/ERF、TGACG基序结合因子(BZIP)、MYC2(BHLH)、ARF、MYB、WRKY和NAC等7种，后两者



是高等植物特有的转录因子家族<sup>[93]</sup>。研究WRKYs转录因子作为MAPK级联反应的重要靶标，在植物对病原菌的抗性中起着关键作用。当病原菌侵入后，SA依赖的WRKY基因会迅速表达并积累，与抗病基因启动子上的W盒（W-box，TTGAC（C/T））特异性结合，启动防御反应，从而形成复杂的WRKY调控网络。苹果中，MKK4-MPK3下游转录因子MdWRKY15、MdWRKY17及MdWRKY100通过调控SA积累增强苹果对炭疽菌的抗性<sup>[70, 94]</sup>。其中，MdWRKY100正向调节苹果对*C. gloeosporioides*的抗性；*C. fructicola*感染可增加感病品种中MdWRKY17蛋白积累，诱导MdMEK4-MdMPK3-MdWRKY17-MdDMR6-SA途径加速SA降解，从而降低果树抗性<sup>[95]</sup>。此外，有研究表明MdWRKY15通过激活SA合成酶MdICS1的表达增强对轮纹病的抗性<sup>[96]</sup>。酯酶/脂肪酶GELP1是MPK3/MPK6及其下游转录因子MdWRKY100的靶标，在提升苹果抵御病原菌侵染中发挥着重要作用<sup>[97]</sup>。Li等研究证实γ-氨基丁酸（GABA）关键合成基因MdGAD1能够与MdWRKY33互作，增强转基因苹果愈伤组织形成和叶片的抗氧化能力，正向调控苹果对*C. gloeosporioides*的抗性<sup>[98, 99]</sup>。此外，MdWRKY31能够与苹果超敏反应蛋白MdHIR4（hypersensitive-induced reaction protein，HIR）相互作用，影响SA信号通路中基因的转录从而调节苹果对葡萄座腔菌*B.dothidea*的抗性的抗性<sup>[100]</sup>。MdWRKY75能够与MdRAC7启动子结合，调节漆酶的生物合成，并在苹果斑点落叶病菌*Alternaria alternata*感染期间增加了木质素的合成<sup>[101]</sup>。最新研究表明，MdVQ10能够与MdWRKY75互作，增强衰老相关基因MdSAG12和MdSAG18的转录，促进叶片损伤引发的衰老进程<sup>[102]</sup>。然而，以上几个转录因子是否在苹果对炭疽菌抗性中也发挥着相同或类似的作用仍有待验证。

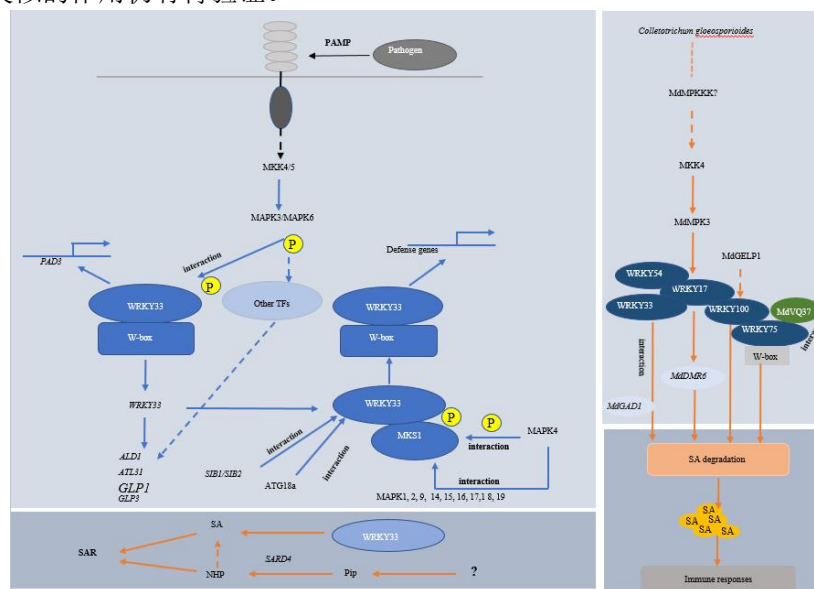


图3 AtwrKY33和MdWRKYs参与MAPK级联反应下游抗病病原菌调控模式图<sup>[94, 95, 97-99, 103]</sup>  
 Fig.3 Schematic model of AtwrKY33 and MdWRKYs involved in downstream pathogenic pathogens of the MAPK cascade

### 3.5 miRNA 参与植物免疫的调控机制

非编码的RNA分为微小RNA（microRNAs, miRNAs）和小干扰RNA（small interfering

RNAs, siRNA) 两大类, miRNA是植物生长发育和胁迫应答中重要的调控因子<sup>[104]</sup>。miRNA可能参与调节病原菌感染中胼胝质沉积过程, 在模式植物拟南芥中, miR773靶向抑制甲基转移酶2 (MET2), 影响胼胝质沉积和ROS累积, 负调控*C. higginsianum*的抗病性<sup>[105]</sup>。Zhang等研究发现, Md-miRln20靶向Md-TN1-GLS负调控苹果对胶孢炭疽菌的侵染<sup>[106]</sup>。此外, Zhang研究证实两种CCR-NB-LRR蛋白MdRNL2和MdRNL6能够形成复合物, 抑制病原菌生长, 提升了苹果树对苹果斑点落叶病菌*A. alternata*的抗性<sup>[107]</sup>, 进一步研究证实其同样能够提升果树对*C. gloeosporioides*的抗性<sup>[108]</sup>。张亚楠等分析了抗感品种中抗病相关miRNA的表达量差异, 预测miR390a、miR482b及miR396b/c/f在苹果被炭疽菌侵染中发挥着重要作用<sup>[109]</sup>。Shen等研究发现Mdm-miR160-MdARF17-MdWRKY33模块能够通过调节活性氧(ROS)调节苹果耐寒性能, 但其对病原菌致病力影响仍未证实<sup>[110]</sup>, 上述结果对果树抗病育种起到重要推动作用。

#### 4 问题与展望

近年来, 在分子生物学和生物信息学的推动下, 苹果和炭疽菌互作方面的研究取得了巨大进展。本文详细阐述了苹果炭疽菌组成、病原菌侵染相关基因及其与寄主互作的研究进展。明确病原菌致病过程及其分子机理, 对苹果炭疽叶枯病的综合防控具有重要意义, 随着各项研究的日益深入, 研究者对炭疽病的防控思路必将有所转变。

炭疽菌对苹果产业造成的危害是巨大的, 由于炭疽菌具有潜伏侵染的特性, 果树病害监测不仅耗时费力, 还存在一定的技术难题。利用传统的化学防控手段, 通过大量使用杀菌剂和杀虫剂等虽能达到对抗病原菌的效果, 但对环境和人身也会造成严重的危害。随着生活水平的不断提高, 健康问题已经逐渐成为关注焦点。目前, 开发生态友好型生物防治策略已经被人们广泛接受和认可, 前景一片光明。开展抗性品种选育是从根本上解决果树炭疽菌防控难的手段, 符合现代农业生产需求<sup>[111, 112]</sup>。解析抗病机制能够为苹果抗病性改良提供重要的理论依据, 是果业科研的重点研究领域。

生物信息学和分子生物学的各种先进技术为作物改良提供了其他途径, 能够极大的缩短果树育种周期, 克服传统实生苗选育耗时长的难题。通过构建苹果高效遗传转化体系进一步开展基因编辑、RNA干扰技术等生物育种技术研究, 培育具有优良性状的苹果新种质或新品种是果树科研工作者努力的方向<sup>[112]</sup>。

总之, 了解苹果与炭疽菌互作的分子机制, 能够为培育抗病品种和创新病害防控策略提供新的见解, 对果树产业健康发展具有重要指导意义。

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